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 FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 13:04:17 ON 26 AUG 2005
L1            22 S GFP (P) DHFR
L2          3435 S IRES OR "RIBOSOMS ENTRY SITE"
L3          6617 S INTRON AND (SPLICE (3W) SITE)
L4        326766 S PROMOTER
L5         53651 S EXPRESSION (3W) (VECTOR OR PLASMID OR POLYNUCLEOTIDE OR CONST
L6          5802 S DICISTRONIC OR BICISTRONIC OR MONOCISTRONIC
L7         13468 S GLUTAMINE (2W) SYNTHET?
L8             3 S L1 AND L6
L9             1 DUP REM L8 (2 DUPLICATES REMOVED)
L10        36853 S CHISHOLM?/AU OR CROWLEY?/AU OR KRUMMEN?/AU OR MENG?/AU
L11           11 S L10 AND L6
L12            4 DUP REM L11 (7 DUPLICATES REMOVED)
L13            1 S L2 AND L3 AND L6
L14            0 S L5 AND L10 AND L1
L15          101 S L5 AND L4 AND L6
L16           48 S GFP AND (DHFR OR L7)
L17            0 S L16 AND L15
L18            0 S L16 AND L10
L19            0 S L3 AND L16
L20            0 S L2 AND L16

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L9 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000161123 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10694794
TITLE: Efficient gene transfer into human cord blood CD34+ cells and the CD34+CD38- subset using highly purified recombinant adeno-associated viral vector preparations that are free of helper virus and wild-type AAV.
AUTHOR: Nathwani A C; Hanawa H; Vandergriff J; Kelly P; Vanin E F; Nienhuis A W
CORPORATE SOURCE: Division of Experimental Hematology, Department of Hematology/Oncology, St Jude Children's Research Hospital, Memphis, TN 38105, USA.
CONTRACT NUMBER: P01HL53749 (NHLBI)
SOURCE: Gene therapy, (2000 Feb) 7 (3) 183-95.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000327
Last Updated on STN: 20021218
Entered Medline: 20000316

AB Recombinant adeno-associated viral (rAAV) vectors have been evaluated for their ability to transduce primitive hematopoietic cells. Early studies documented rAAV-mediated gene expression during progenitor derived colony formation in vitro, but studies examining genome integration and long-term gene expression in hematopoietic cells have yielded conflicting results. Such studies were performed with crude vector preparations. Using improved methodology, we have generated high titer, biologically active preparations of rAAV free of wild-type AAV (less than 1/107particles) and adenovirus. Transduction of CD34+ cells from umbilical cord blood was evaluated with a **bicistronic** rAAV vector encoding the green fluorescent protein (**GFP**) and a trimetrexate resistant variant of dihydrofolate reductase (**DHFR**). Freshly isolated, quiescent CD34+ cells were resistant to transduction (less than 4%), but transduction increased to 23 +/- 2% after 2 days of cytokine stimulation and was further augmented by addition of tumor necrosis factor alpha (51 +/- 4%) at a multiplicity of infection of 106. rAAV-mediated gene expression was transient in that progenitor derived colony formation was inhibited by trimetrexate. Primitive CD34+ and CD34+, CD38- subsets were sequentially transduced with a rAAV vector encoding the murine ecotropic receptor followed by transduction with an ecotropic retroviral vector encoding **GFP** and **DHFR**. Under optimal conditions 41 +/- 7% of CD34+ progenitors and 21 +/- 6% of CD34+, CD38- progenitors became trimetrexate resistant. These results document that highly purified rAAV transduce primitive human hematopoietic cells efficiently but gene expression appears to be transient. Gene Therapy (2000) 7, 183-195.

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L13 ANSWER 1 OF 1 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
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ACCESSION NUMBER: 1999158596 EMBASE
TITLE: Efficient **bicistronic** expression of cre in mammalian cells.
AUTHOR: Gorski J.A.; Jones K.R.
CORPORATE SOURCE: K.R. Jones, Dept. Mol., Cellular Dev. Biology, University of Colorado, Boulder, CO 80309, United States. krjones@stripe.colorado.edu
SOURCE: Nucleic Acids Research, (1 May 1999) Vol. 27, No. 9, pp. 2059-2061.
Refs: 25
ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19990520
Last Updated on STN: 19990520

AB Cre recombinase-mediated DNA recombination is proving to be a powerful technique for the generation of mosaic mutant mice. To develop this technology further, we have altered the cre gene to enhance its expression in mammalian cells and have tested its efficiency of expression in a **bicistronic** message. Using a transient transfection assay, we found that the extension of a eukaryotic translation initiation consensus sequence, the insertion of two N-terminal amino acids, and the mutation of a cryptic **splice** acceptor **site** did not detectably alter Cre recombinase activity. The addition of either of two introns resulted in an .apprx.2-fold increase in recombination frequency. We then tested the relative efficacy of Cre-mediated recombination in several **bicistronic** messages having the encephalomyocarditis virus internal ribosome entry site (**IRES**). Recombination frequencies were only reduced 2-fold relative to a comparable **monocistronic** cre gene. The latter results indicate that it will be possible to generate transgenic mouse strains having tissue-specific expression of the Cre recombinase through integration of an **IRES**-cre gene without disabling the targeted gene.

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